## Materials and methods

### *Bacterial isolates and antimicrobial susceptibility testing*

Species identification of isolates was carried out using a Vitek® 2 system (bioMérieux, France), 16S rRNA gene sequencing and 16S-23S rRNA gene intergenic spacer sequencing (1). Antimicrobial susceptibility testing was performed by microbroth dilution according to the Clinical and Laboratory Standards Institute (CLSI) (2). Etest® strips (bioMérieux) for imipenem and meropenem were selected for drug susceptibility tests. Carbapenemase activity was assessed with Etest® metallo-β-lactamases (imipenem/imipenem + EDTA).

### *Molecular detection of β-lactamase production*

Carbapenemase, ESBL and AmpC resistance genes (*bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{OXA-2 group}}$, *bla*$_{\text{OXA-10 group}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{VEB}}$, *bla*$_{\text{PSEgroup}}$, *bla*$_{\text{CMY-2}}$ and *bla*$_{DHA\text{-}1}$) were screened by PCR (3-5). Aminoglycoside resistance genes (*bla*$_{\text{aac(6')/aph(2")}}$, *bla*$_{\text{aac(6')-Ib}}$, *bla*$_{\text{aac(3)-I}}$ and *bla*$_{\text{armA}}$) were also detected by PCR (6). Positive PCR results were confirmed by sequencing. The nucleotide and deduced protein sequences were searched against the National Center for Biotechnology Information (NCBI) database.

### *Transconjugation assays and Southern blot analysis*

Horizontal transfer capability of the *bla*$_{\text{NDM}}$ gene was assessed by broth and filter mating using a standard *E. coli* J53 azide-resistant strain as the recipient. MacConkey

agar containing 100 mg/L sodium azide and 0.5 mg/L meropenem was used to select for *E. coli* J53 transconjugants (7). Putative transconjugants were confirmed by detection of $bla_{NDM}$ with PCR as described above.

Moreover, three enteric pathogens including *Shigella sonnei* Ss046, *Shigella flexneri* 2a 301 and *Salmonella Serotype Paratyphi* A CMCC 50973 (stored in our laboratory) were used in the conjugation to detect whether or not resistant genes could transform into enteric pathogens. The transconjugants were separated by immunomagnetic beads (IMBS, Estapor Merck, France) separation techniques for three enteric pathogens, then selected on SS and MacConkey agar mediums (BD DIFCO, USA) with 0.5 mg/L meropenem. All of the transconjugants were identified by PCR for $bla_{NDM}$.

Genomic DNA agarose blocks from clinical isolates and transconjugant strains were digested with the S1 endonuclease (Takara). DNA fragments were separated by PFGE with a CHEF-DR III apparatus (Bio-Rad, Hercules, USA) for 20 h at 6 V/cm and 14 °C, with initial and final pulse time of 5 s and 30 s, respectively. Southern blot analysis was performed to locate the $bla_{NDM}$ genes using specific $bla_{NDM}$ digoxigenin-labeled probes (Roche) (8). *Klebsiella pneumoniae* ATCC BAA-2146 was used as a positive control and *E. coli* J53 was used as a negative control.

***Plasmid DNA sequencing, annotation and analysis***

Plasmid DNA was extracted using the BAC/PAC DNA Kit (Omega Biotek, Norcross, GA, USA) according to the manufacturer's protocol. Complete DNA sequences of plasmids were obtained using the Ion Torrent™ sequencing platform (9). Raw reads were

firstly assembled into contigs using Newbler version 2.9, followed by gap filling by local assembly. To ensure accuracy, complete genomes were confirmed and revised by mapping the raw reads onto the assembled genomes. Each assembled genome was annotated with the Rapid Annotations using Subsystems Technology (RAST) server and verified with the Basic Local Alignment Search Tool (BLAST) against the non-redundant NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (10).

### *Cloning $bla_{NDM-14}$ and $bla_{NDM-1}$*

To compare the relative contributions of NDM-1 and NDM-14 to carbapenem resistance, the entire open reading frame (primers NDM-F: 5′-CGGGATCCATGGAATT GCCCAATATTATG-3′ and NDM-R: 5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCA T-3′) and the complete gene with its native promoter (primers NP-NDM-F: 5′-CGGGATCCCACCTCATGTTTGAATTCGC-3′ and NP-NDM-R: 5′-CCCAAGCTTC TCTGTCACATCGAAATCGC-3′) were amplified and cloned into the corresponding sites of pHSG398 (Takara Bio) (11, 12). *E. coli* DH5α cells were transformed with pHSG398-NDM-1, pHSG398-NDM-14, pHSG398-NP-NDM-1, and pHSG398-NP-ND M-14 to determine β-lactam MIC. The *A. lwoffii* clinical isolate JN49-1 and *A. lwoffii* JN247 genomes were used as PCR templates and gene cloning results were confirmed by sequencing.

### *Kinetic Study, Native PAGE and In-Gel Activity Staining*

The open reading frames of NDM-1 and NDM-14 without signal peptide regions were cloned into the expression vector pET28a using the primer set *Bam*HI-TEV-NDM-

（5′-CGGGATCCGAAAACCTGTATTTCCAAGGCCAGCAAATGGAAACTGGCGA C-3′） and *Xho*I-NDM-R (5′-CCGCTCGAGTCAGCGCAGCTTGTCGGCCATG-3′) (12). *E. coli* BL21 (DE3) was used to express the the recombinant NDM proteins, which was purified by using the nickel-nitrilotriacetic acid (Ni-NTA) agarose according to the manufacturer's instruction (Qiagen). His tags were cleaved using the TurboTEV protease (Accelagen, San Diego, CA), and the tags and protease were removed by an additional passage over Ni-NTA agarose. The purity of the recombinant NDM proteins was estimated up to 90% by SDS-PAGE. The protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific, USA). The hydrolysis rates were monitored in 50mM phosphate buffer (pH7.0) at 37°C, using SpectraMax 190 microplate reader (Molecular Devices, USA). The *K*m, *k*cat values and the *k*cat/*K*m ratios were determined using a Lineweaver-Burk plot. Wavelengths and extinction coefficients for β-lactam substrates have been reported previously (13-15). Three individual experiments were performed to determine the *K*m and *k*cat values.

Native PAGE of NDM-1 and NDM-14 was performed according to protocols reported by Hu et al (16). The purified untagged protein were used in the native gel, which was immersed in a 0.5 mg/ml nitrocefin solution (Oxoid Ltd., Basingstoke, England) 10 min at 37°C to visualize the β-lactamase active band. The final loading amount of NDM proteins was 0.125μg, 0.25μg, 0.5μg and 1μg respectively.

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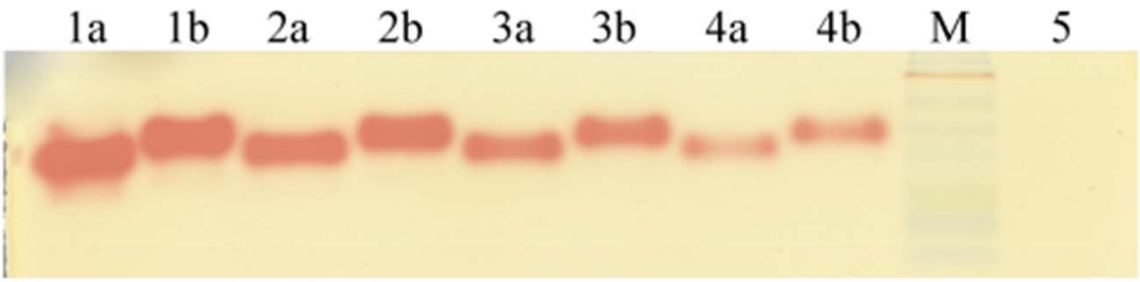
Supplemental Table 1. Primer pairs used for β-lactamase screening

| Primer pairs | Sequence | β-Lactamase detected | Product size (bp) |
|---|---|---|---|
| 1 | 5'-GGAATAGAGTGGCTTAAyTCTC-3'<br>5'-CCAAACyACTACGTTATCTkGAG-3' | IMP | 188 |
| 2 | 5'-GATGGTGTTTGGTCGCATA-3'<br>5'-CGAATGCGCAGCACCAG-3' | VIM | 390 |
| 3 | 5'-CTTGCTGCCGCTGTGCTG-3'<br>5'-GCAGGTTCCGGTTTTGTCTC-3' | KPC | 489 |
| 4 | 5'-GATGTGTCATAGTATTCGTCGT-3'<br>5'-TCACAACAACTAAAAGCACTGT-3' | OXA-23-like | 1058 |
| 5 | 5'-ATGAAAAAATTTATACTTCCTATATTCAGC-3'<br>5'-TTAAATGATTCCAAG ATTTTCTAGC-3' | OXA-24-like | 828 |
| 6 | 5'-TAATGCTTTGATCGGCCTTG-3'<br>5'-TGGATTGCACTTCATCTTGG-3' | OXA-51-like | 353 |
| 7 | 5'-AAGTATTGGGGCTTGTGCTG-3'<br>5'-CCCCTCTGCGCTCTACATAC-3' | OXA-58-like | 599 |
| 8 | 5'-TACAAGGGATTCGGCATCG-3'<br>5'-TAATGGCCTGTTCCCATGTG-3' | SIM | 570 |
| 9 | 5'-CCTACAATCTAACGGCGACC-3'<br>5'-TCGCCGTGTCCAGGTATAAC-3' | SPM | 648 |
| 10 | 5'-CTTGTAGCGTTGCCAGCTTTA-3'<br>5'-CAGCCCAAGAGCTAATTGAGG-3' | GIM | 562 |
| 11 | 5'-TGGTTATGCGTTATATTCGCC-3'<br>5'-GGTTAGCGTTGCCAGTGCT-3' | SHV | 868 |
| 12 | 5'-TCCGCTCATGAGACAATAACC-3'<br>5'-TTGGTCTGACAGTTACCAATGC-3' | TEM | 931 |
| 13 | 5'-TCTTCCAGAATAAGGAATCCC-3'<br>5'-CCGTTTCCGCTATTACAAAC-3' | CTX-M | 909 |
| 14 | 5'-AAGAAACGCTACTCGCCTGC-3'<br>5'-CCACTCAACCCATCCTACCC-3' | OXA-2 group | 478 |
| 15 | 5'-GTCTTTCGAGTACGGCATTA-3'<br>5'-ATTTTCTTAGCGGCAACTTAC-3' | OXA-10 group | 720 |
| 16 | 5'-ATGAATGTCATCACAAAATG-3'<br>5'-TCAATCCGGACTCACT-3' | PER | 927 |
| 17 | 5'-ATGCGCTTCATTCACGCAC-3'<br>5'-CTATTTGTCCGTGCTCAGG-3' | GES | 864 |
| 18 | 5'-CGACTTCCATTTCCCGATGC-3'<br>5'-GGACTCTGCAACAAATACGC-3' | VEB | 644 |

| | | | |
|---|---|---|---|
| 19 | 5'-ACCGTATTGAGCCTGATTTA-3'<br>5'- ATTGAAGCCTGTGTTTGAGC-3′ | PSE group (PSE-1, PSE-4, CARB-3) | 321 |
| 20 | 5'-TTTCTCCTGAACGTGGCTGGC-3'<br>5'-TGGCCAGAACTGACAGGCAAA-3' | CMY-2 | 462 |
| 21 | 5'-AACTTTCACAGGTGTGCTGGGT-3'<br>5'-CCGTACGCATACTGGCTTTGC-3' | DHA-1 | 405 |




**Supplemental Figure 1**. Native page and in-gel activity staining by nitrocefin. Lane 1a, 2a, 3a and 4a were NDM-1; Lane 1b, 2b, 3b and 4b were NDM-14. M: protein maker, 5: blank control. Protein amount of lane 1a and 1b was 1μg, lane 2a and 2b was 0.5μg, lane 3a and 3b was 0.25μg, lane 4a and 4b was 0.125μg.